INVERSE-DETECTED NMR OF CIGUATOXIN: QUATERNARY CARBON LOCATIONS CONFIRMED IN CTX-1

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Short-range (HMQC, $^{1}J_{CH}$) and long-range (HMBC, $^{2,3}J_{CH}$) 2-dimensional inverse-detected heteronuclear nuclear magnetic resonance spectra of 0.45mg of ciguatoxin-1 are shown. These spectra provide independent support for the structure proposed for ciguatoxin-1 and confirm the ^{13}C assignments and the location of the two quaternary carbons in ciguatoxin-1. The presence of four ether linkages was also confirmed from the HMBC experiment.

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A major advance in ciguatera research was the determination of the structure of ciguatoxin (Murata et al.,1989,1990). Ciguatoxin-1 (=CTX-1, Fig.1) is the most toxic ciguatoxin isolated to-date (Lewis et al.,1991) and is dominant in ciguateric fish flesh (Lewis & Sellin,1992). CTX-1 is probably responsible for the clinical syndrome that follows consumption of ciguateric fish; especially carnivorous species.

The structure of CTX-1 was proposed on the basis of one-dimensional ^{1}H NMR and nOe spectroscopy, two-dimensional homonuclear scalar coupled spectroscopy ($^{2\text{-}6}J_{HH}$) and mass spectroscopy (Murata et al.,1989,1990). Short-range (one bond) inverse-detected heteronuclear experiments (HMQC) supported the structure proposed for CTX-1 (Murata et al.,1992). The absolute stereochemistry of CTX-1 (Fig.1) has been proposed (Suzuki et al.,1991). Short-range (HMQC) and long-range (HMBC) inverse-detected spectra of CTX-1, determined using the method of Martin & Crouch (1991), provide independent support for this structure, including confirmation of the location of the two quaternary carbons in the molecule.

METHODS

Ciguatoxin-1 (CTX-1)

CTX-1 was purified in 1991 (Lewis et al., 1991). NMR experiments were performed on a 0.45mg sample of CTX-1 in 0.45ml of pyridine-d_5 (99.96%, Cambridge Isotope Laboratories).

Nuclear Magnetic Resonance (NMR) Spectroscopy

The formation of heteronuclear multiple quantum coherence between protons and ^{13}C nuclei provides a powerful tool for molecular structure determination. Two dimensional experiments

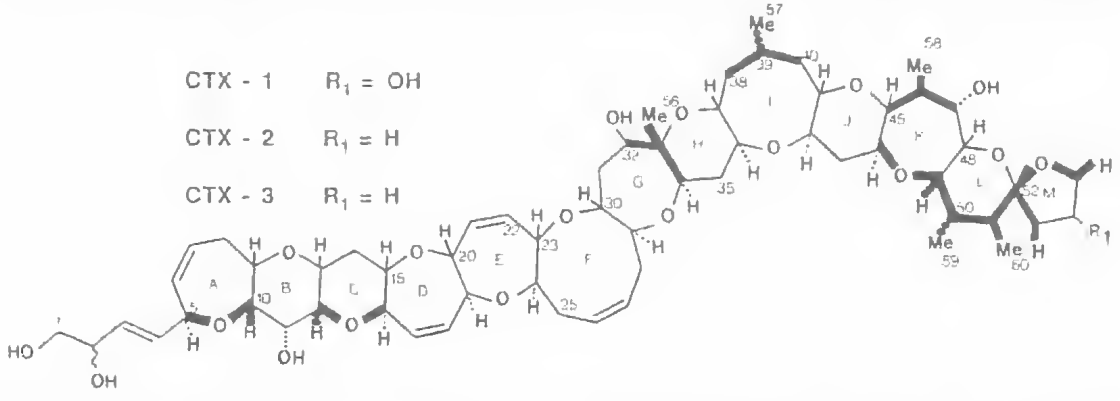

FIG.1.Structure of ciguatoxin-1 (R_1 = OH) proposed by Murata et al. (1990). Connectivities confirmed from the HMBC spectrum are shown bolded.

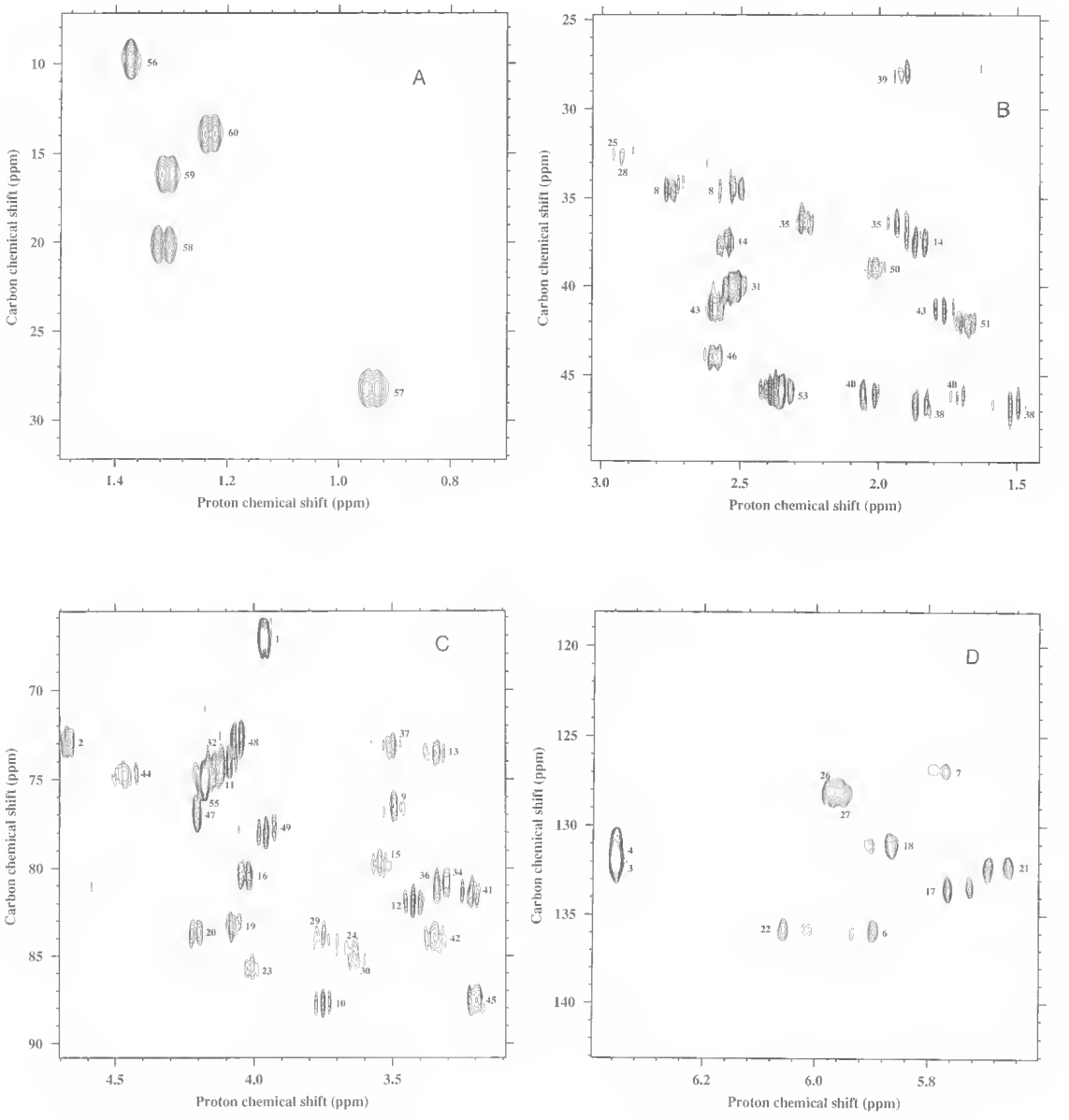

FIG.2. HMQC spectrum of ciguatoxin-1 (CTX-1) at 400 MHz in pyridine-d_5 (30°C). $^1J_{CH}$ clustered into four regions shown in detail in panels A to D. Carbon number of the carbons giving rise to $^1J_{CH}$ are labelled according to the structure of CTX-1 (Fig.1). Data were zero filled to give a matrix consisting of 4 K x 1 K points.

provide extensive proton to carbon connectivities, both direct via one bond scalar coupling ($^1J_{CH}$) with the HMQC experiment and long range via 2 and 3 bond scalar couplings ($^{2,3}J_{CH}$) with the HMBC. The long-range experiment allows correlations across and to quaternary carbons (i.e. carbons without attached protons) and across heteroatoms (e.g. oxygens). Whether short- or long-range correlations are determined depends on the evolutionary manipulation of heteronuclear coherences according to the value of the respective heteronuclear coupling constants (~140Hz for one bond couplings and 5–10 Hz for 2 and 3 bond couplings). The sensitivity of these experiments can be maximised if the generated heteronuclear multiple quantum

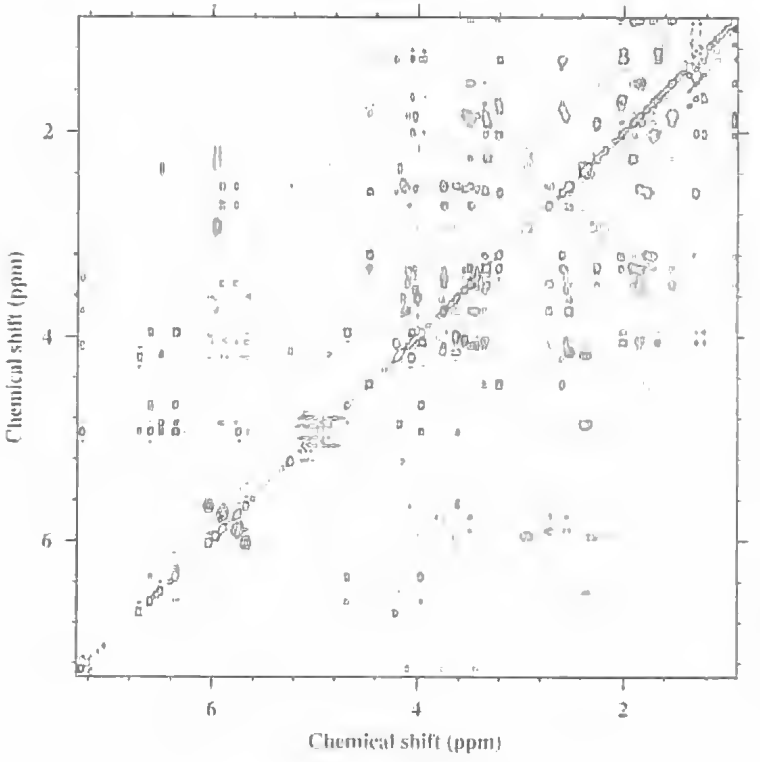

FIG.3.HOHAHA spectrum of ciguatoxin-1 (CTX-1) at 500MHz in pyridine-d_5 (30°C). Chemical exchange between hydroxyl protons of CTX-1 and H_2O was detected as direct and relayed cross-peaks from each hydroxyl proton to the water resonance at 4.94ppm (mixing time 55msec). Data were zero filled to give a matrix consisting of 4 K x 1 K points.

coherences are converted to observable proton signals, rather than using magnetisation from the less sensitive heteronucleus, in the so-called "inverse" or proton detected correlation experiment. The one-bond correlation variant of this experiment is known as the inverse-detected HMQC and the somewhat less sensitive long-range variant as the inverse-detected heteronuclear multiple bond correlation (HMBC) experiment. These procedures were applied to the ciguatoxins as described below.

The short-range ($^1J_{CH}$) inverse-detected (HMQC experiment) two-dimensional NMR spectrum of CTX-1 was obtained at 400MHz on a Bruker AMX-400 at 30°C (206 t_1 values, each with 2048 points (512 scans averaged) over a spectral width of 4000Hz). The long-range ($^{2,3}J_{CH}$) inverse-detected (HMBC) two-dimensional NMR experiment on CTX-1 was performed at 500MHz on a Bruker AMX-500 at 30°C (495 t_1 values, each with 2048 points (96 scans averaged) over a spectral width of 4310Hz) and was optimised for 8.3Hz couplings. The two- dimensional homonuclear Hartman Hahn experiment (HOHAHA) was performed according to Bax & Davis (1985) at 500MHz on an AMX-500 at 30°C (246 t_1 values, each with 2048 points (88 scans averaged) over a spectral width of 4505Hz). No water suppression was used for these experiments. The NMR data were processed using FTTOOL and spectra analysed on a SUN SPARCstation-2 also using this software. For each 2-D experiment, a gaussian deconvolution with line broadening was applied to t_2 data (data zero filled to give 4K points in F2) and a Hamming filter applied to t_1 data (zero-filled to give 1K points in F1) to obtain optimal signal to noise in each dimension. Chemical shifts are given in ppm downfield of the pyridine resonance (^1H at 7.21ppm and ^{13}C at 123.5ppm).

RESULTS

The HMQC spectrum of CTX-1 (Fig.2) detected all $^1J_{CH}$ except those associated with the proton resonances at 4.86 ppm which were obscured by the H_2O resonance which was not suppressed in this experiment. This spectrum allowed us to independentlydetermine the ^{13}C chemical shifts for each carbon on CTX-1 and the ^1H chemical shifts of the attached proton (Table 1). These data correspond to the assignment of Murata et al. (1992), with the minor exception of carbon 36 to which we assign a ^{13}C chemical shift of 81.0ppm, as opposed to 83.69ppm. These data confirm all methines in CTX-1 and the assignment of the double bond in the flexible portion of CTX-1 (ring F). In addition, all carbons assigned as attached to oxygen had ^{13}C chemical shifts characteristic of such a chemical environment. The ^{13}C assignments were confirmed by careful comparison of the coupling patterns of protons observed in the HMQC, HOHAHA and reference

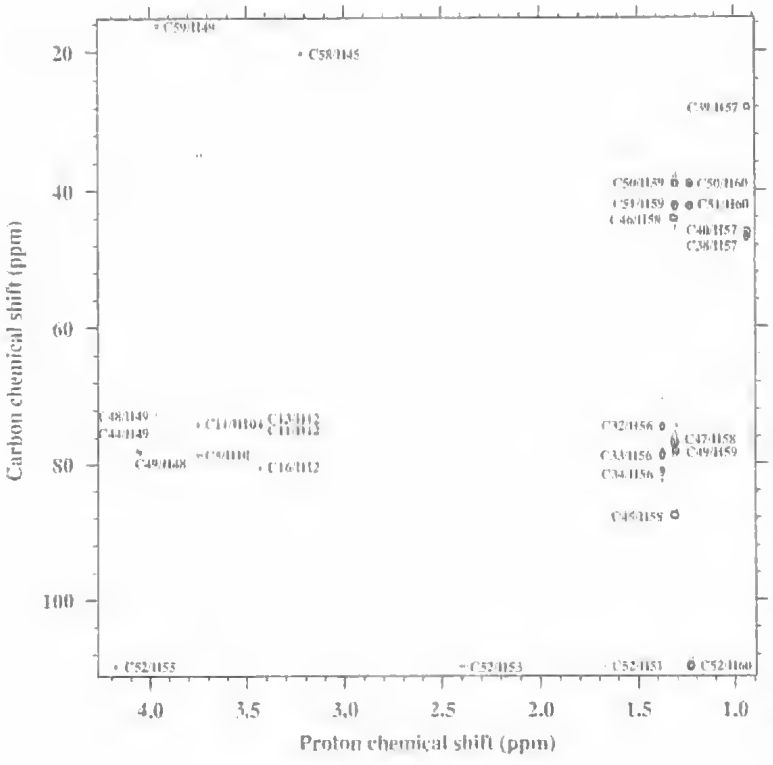

FIG.4.HMBC spectrum of ciguatoxin-1 (CTX-1) at 500 MHz in pyridine-d_5 (30°C). $^{2,3}J_{CH}$ are labelled according to the proposed structure of CTX-1 (Fig. 1). Data were zero filled to give a matrix consisting of 4 K x 1 K points.

1-D ^{1}H NMR spectra. All ^{1}H chemical shifts assigned from the HMBC experiment overlapped the chemical shifts assigned from the HOHAHA experiment also obtained at 30°C (Fig.3). In addition to scalar coupled connectivities, the HOHAHA (55msec mixing time) also detected the chemical exchange between hydroxyl protons of CTX-1 and residual H_2O in the solvent. Similar exchange was detected previously for CTX-2 (Lewis et al.,1993). Such signals provide a useful means of identifying the exchangeable hydroxyl protons in molecules.

The HMBC spectra of CTX-1 (Fig.4) includes $^{2,3}J_{CH}$ for all methyl protons. Fortunately, the two quaternary carbons in CTX-1 (carbons 33 and 52) were within three bonds of a methyl, allowing us to confirm unambiguously the ^{13}C chemical shift (Table 1) and location (Fig.1) of these carbons. Most other long-range couplings expected for the proposed structure of CTX-1 were not detected, at least in part owing to the small quantity of CTX-1 available (experiments were performed on a 1 mM solution). However, we were able to confirm the location of four ether linkages by this experiment (Figs 1,4).

DISCUSSION

We obtained HMQC and HMBC spectra of a 1mM solution of CTX-1 in pyridine-d_5 at 30°C. The HMQC spectrum independently confirmed the ^{13}C assignments of CTX-1 given by Murata et al. (1992). The HMBC spectra confirmed the position of carbons 33 and 52, the two quaternary carbons present in CTX-1 (Fig.1). This spectrum also confirmed the location of four of the 13 ether linked rings (Fig. 1). The location of these quaternary carbons and 12 of the 13 ether linkages was previously inferred from ^{13}C chemical shifts and one-dimensional nOe experiments (Murata et al.,1990).

The HMBC experiment detected all carbons two or three bonds from methyl protons but owing to the relatively poor signal to noise of this experiment (compared to the HMQC experiment) it was not sufficiently sensitive to detect many of the $^{2,3}J_{CH}$ couplings. These couplings may also be either larger or smaller than 8.3Hz, the coupling size for which the experiment was optimised. In conclusion, our data support the structure proposed originally for CTX-1 by Murata et al. (1989).

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TABLE 1. ^{13}C and ^{1}H NMR chemical shifts for CTX-1 at 30°C (400MHz, pyridine-d_5) from the HMQC experiment.

Position	^{13}C (ppm)	^{1}H (ppm)	Position	^{13}C (ppm)	^{1}H (ppm)
1	67.1	3.96	31	40.0	2.52,2.52
2	72.9	4.68	32	74.5	4.16
3	132.0	6.36	33	78.6[b]	-
4	131.0	6.36	34	80.8	3.31
5	78.0[a]	4.86	35	36.4	1.92, 2.26
6	136.0	5.91	36	81.0	3.34
7	126.8	5.78	37	73.1	3.50
8	34.6	2.54, 2.73	38	46.8	1.54, 1.84
9	76.6	3.50	39	27.8	1.91
10	87.8	3.75	40	46.1	1.71, 2.03
11	74.1	4.10	41	81.5	3.21
12	82.0	3.43	42	84.0	3.35
13	73.6	3.34	43	41.4	1.78, 2.59
14	37.5	1.85, 2.56	44	74.8	4.47
15	79.7	3.55	45	87.6	3.20
16	80.5	4.03	46	44.0	2.59
17	133.6	5.74	47	77.0	4.21
18	131.1	5.89	48	72.6	4.06
19	83.3	4.07	49	77.9	3.96
20	83.6	4.21	50	39.0	2.01
21	132.3	5.67	51	42.2	1.68
22	135.9	6.04	52	109.7[b]	-
23	85.7	4.02	53	45.9	2.34, 2.40
24	85.0	3.64	54	70.7[a]	4.86
25	32.5	~2.2, 2.96	55	75.1	4.18, 4.18
26	128.2	5.97	56	9.7	1.37
27	128.2	5.96	57	28.3	0.94
28	32.7	~2.3, 2.93	58	20.2	1.32
29	83.9	3.76	59	16.2	1.31
30	85.4	3.63	60	13.9	1.23

[a] 1H chemical shifts at 4.86ppm (2 protons) were obscured by the water resonance (^{13}C values from Murata et al.,1992).

[b] ^{13}C chemical shift values for quaternary carbons from the HMBC experiment at 30°C (500 MHz, pyridine-d_5).

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